FOR THE RECORD

Stabilization of membranes upon interaction of amphipathic polymers with membrane proteins

MARTIN PICARD,[1] CAROLINE DUVAL-TERRIÉ,[2] EMMANUELLE DÉ,[2] AND PHILIPPE CHAMPEIL[1]

[1]Unité de Recherche Associée 2096 (Centre National de la Recherche Scientifique et Commissariat à l'Energie Atomique) & Section de Biophysique des Fonctions Membranaires, Département de Biologie Joliot-Curie, CEA Saclay, 91191 Gif-sur-Yvette cedex, France, and Laboratoire de Recherche Associé 17V & Institut Fédératif de Recherches 46, Université Paris Sud, France
[2]Unité Mixte de Recherche 6522 (Centre National de la Recherche Scientifique), Université de Rouen, 76821 Mont-Saint-Aignan cedex, France

Abstract

Amphipathic polymers derived from polysaccharides, namely hydrophobically modified pullulans, were previously suggested to be useful as polymeric substitutes of ordinary surfactants for efficient and structure-conserving solubilization of membrane proteins, and one such polymer, $18C_{10}$, was optimized for solubilization of proteins derived from bacterial outer membranes (Duval-Terrié et al. 2003). We asked whether a similar ability to solubilize proteins could also be demonstrated in eukaryotic membranes, namely sarcoplasmic reticulum (SR) fragments, the major protein of which is SERCA1a, an integral membrane protein with Ca^{2+}-dependent ATPase and Ca^{2+}-pumping activity. We found that $18C_{10}$-mediated solubilization of these SR membranes did not occur. Simultaneously, however, we found that low amounts of this hydrophobically modified pullulan were very efficient at preventing long-term aggregation of these SR membranes. This presumably occurred because the negatively charged polymer coated the membranous vesicles with a hydrophilic corona (a property shared by many other amphipathic polymers), and thus minimized their flocculation. Reminiscent of the old Arabic gum, which stabilizes Indian ink by coating charcoal particles, the newly designed amphipathic polymers might therefore unintentionally prove useful also for stabilization of membrane suspensions.

Keywords: membrane protein; solubilization; amphiphilic polysaccharide; pullulan; sarcoplasmic reticulum; corona

Amphipathic linear polymers, with randomly distributed polar groups and nonpolar side chains, were proposed to be useful tools for the handling of membrane proteins in isolated form, thanks to multipoint attachment of the polymer nonpolar side chains onto the transmembrane protein hydrophobic surface (Tribet et al. 1996; Popot et al. 2003). The ability of such amphipathic polymers to directly solubilize membranes, however, is dependent on a number of factors (e.g. Thomas and Tirrell 1992; Tribet 1998 and references herein). For instance, although certain polyacrylate-based amphipathic polymers have been shown to efficiently keep membrane proteins soluble *after* their extraction from membranes by detergents, they proved to be poor membrane solubilizers per se (e.g., Champeil et al. 2000; Nagy et al. 2001; Ladavière et al. 2002); in contrast, other

amphipathic polymers were reported to be endowed with significant efficiency for the solubilization of membrane proteins: This was the case for hydrophobically modified pullulans, when added to bacterial outer membrane proteins (e.g., Duval-Terrié et al. 2003). To allow for comparison of the properties of the two classes of polymer on the same biological material, we complemented our initial study of the ability of hydrophobically modified polyacrylate to solubilize sarcoplasmic reticulum (SR) membranes (Champeil et al. 2000) with the present study of the ability of a modified pullulan, $18C_{10}$ (Duval-Terrié et al. 2003), to solubilize the same SR membranes.

Results and Discussion

In the absence of detergent, interaction with intact SR membranes of the modified pullulan $18C_{10}$ does not *solubilize these membranes but minimizes their time-dependent flocculation.*

We asked whether incubation of intact SR membranes with the modified pullulan $18C_{10}$ would lead to membrane solubilization in the absence of any detergent. To answer this question, we simply measured after dilution the turbidity of appropriate samples, incubated with modified pullulan for up to a few days. As shown in Figure 1, A or B, the spectrum of such samples reveals superimposition of both the expected UV absorption of the membrane proteins and the turbidity spectrum characteristic of light scattering by small particles (SR vesicles are known to be 60–200 nm in diameter). Over a 4-d incubation period of SR membranes with the modified pullulan, used either at a low (Fig. 1A) or a higher (Fig. 1B) concentration, this composite spectrum essentially did not change; this was also true (data not shown) in the case of SR membrane 4-d incubation with another charged and hydrophobically modified polymer, amphipol A8–35 (Tribet et al. 1996), derived from polyacrylate and previously studied over shorter periods with the same result (Champeil et al. 2000). The apparent discrepancy between the present results and those in Duval-Terrié et al. (2003), where different biological membranes were used, is probably simply another example of the well-known fact that different types of membranes are solubilized to different extents by a given surfactant (e.g., the microdomains known as "rafts" in plasma membranes, compared with the less specialized areas of the membrane; see Schuck et al. 2003). Originally, $18C_{10}$ was, in fact, optimized with respect to specific solubilization of bacterial outer membranes.

The disappointing result that modified pullulan $18C_{10}$ is not efficient for solubilization of SR membranes, however, regains interest upon comparison with what happened to SR membranes in the absence of polymer (Fig. 1C) in the same experiment: In the absence of detergent or polymer, the turbidity of the membranes increased to a large extent over
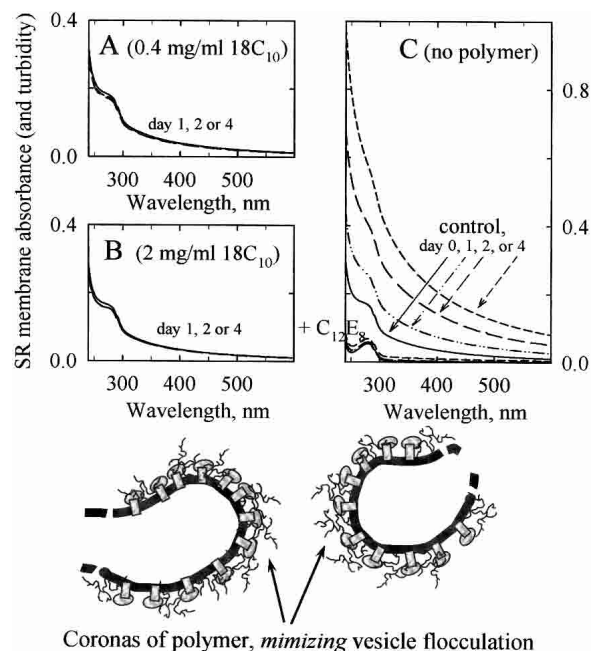

Figure 1. The modified pullulan $18C_{10}$ does *not* solubilize SR membranes, but *prevents* their flocculation in the absence of detergent. SR membranes were suspended at 1 mg protein/mL in a buffer consisting of 100 mM KCl, 1 mM Mg^{2+}, 0.1 mM Ca^{2+}, and 50 mM Tes-Tris at pH 7.5 (at 20°C). An aliquot of this suspension was supplemented with 2 mg/mL $C_{12}E_8$ (*C*), while two other aliquots were supplemented with either 0.4 (*A*) or 2 mg/mL (*B*) of the modified pullulan $18C_{10}$, in the absence of $C_{12}E_8$; the rest was kept as control (also shown in *C*). All samples were left in the cold room (at about 6°C). After various periods over 4 d (15 h, 39 h, or 89 h), 100 μL of the different samples were diluted into 2 mL of the same buffer, and turbidity (and absorption) properties were examined in the UV and visible region. (*Bottom*) Cartoon illustrating the suggested coronas of polymers, coating the membranes.

days, revealing an intrinsic tendency of the membranes for long-term aggregation or flocculation (in the presence of detergent, the typical UV absorbance of membrane proteins of course remained visible, but turbidity vanished, as expected; see bottom spectra in Fig. 1C). Thus, the modified pullulan, even at low concentration (1:2.5 w/w, relative to SR membranes), in fact efficiently *prevents* the intrinsic tendency for flocculation. After a whole week in the cold room, the residual ATPase activity of the membranes, as tested in the presence of excess $C_{12}E_8$, was also preserved better when the membranes had been incubated with the lower concentration of pullulan (or with 2 mg/mL A8-35) than when it had been incubated either with the larger concentration of pullulan or in the absence of polymer (data not shown). Thus, moderate amounts of modified pullulan are sufficient to keep the SR membranes stable.

This property can easily be understood by realizing that the polymers, by adsorbing onto the membranes, form hydrophilic coronas (see cartoon at bottom of Fig. 1) (Ueda et al. 1998) which probably minimize vesicle flocculation,

very much in the same manner as Arabic gum stabilizes Indian ink by coating the carbon droplets suspended in water and preventing them from aggregating and falling down to the bottom of the vessel (de Gennes and Badoz 1994; Currie et al. 2003). This is a very general mechanism, probably applicable to many types of membranes and polymers. Modified pullulans were originally not designed for this purpose, but their antiflocculation properties might prove to be an unexpected benefit of the use of these polymers, for those in the membrane biology field who are interested in keeping membranes in nonaggregated form over long periods. Outside this field, it is, in fact, also of interest that liposomes coated with polysaccharide-derived polymers were found particularly stable carriers for drug targeting (e.g., Sunamoto et al. 1992; for review, see Sihorkar and Vyas 2001): In addition to other factors like recognition by target cells or liposome shielding from opsonization or enzymatic degradation by serum proteins, polysaccharide polymers might endow the coated liposomes with a diminished tendency for flocculation.

Materials and methods

The carboxymethylpullulan $18C_{10}$ (Scheme 1) was synthetized as described previously (Duval-Terrié et al. 2003). This polymer, with an average molecular mass of 30 kDa, is highly charged (on average, 0.91 ungrafted carboxymethyl group and 0.18 hydrophobic chain per anhydroglucose unit; C10 refers to the decyl linear alkyl group used for grafting). Polymer stock solutions were 50 mg/mL in water. SR vesicles were prepared as previously described (Champeil et al. 1985). The nonionic detergent $C_{12}E_8$ was obtained from Nikko.
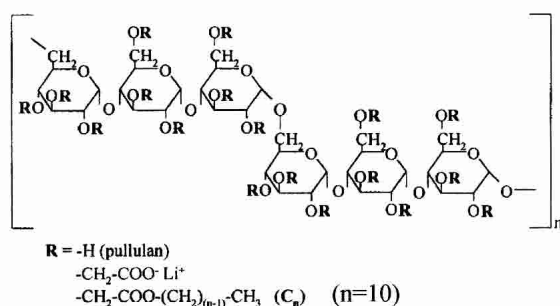
Scheme 1. The hydrophobically modified pullulan $18C_{10}$.

Turbidity (and absorbance) spectra of SR suspensions were measured with a diode array HP 8453 spectrophotometer. Samples in the temperature-regulated cuvette were stirred continuously.

Acknowledgments

References

Champeil, P., Guillain, F., Vénien, C., and Gingold, M.P. 1985. Interaction of magnesium and inorganic phosphate with calcium-deprived sarcoplasmic reticulum adenosinetriphosphatase, as reflected by organic solvent induced perturbation. *Biochemistry* **24:** 69–81.

Champeil, P., Menguy, T., Tribet, C., Popot, J.-L., and le Maire, M. 2000. Interaction of amphipols with sarcoplasmic reticulum Ca²⁺-ATPase. *J. Biol. Chem.* **275:** 18623–18637.

Currie, E.P.K., Norde, W., and Cohen-Stuart, M.A. 2003. Tethered polymer chains: Surface chemistry and their impact on colloidal and surface properties. *Adv. Colloid Interface Sci.* **100–102:** 205–265.

de Gennes, P.G. and Badoz, J. 1994. *Les objets fragiles.* Plon, Paris.

Duval-Terrié, C., Cosette, P., Molle, G., Muller, G., and Dé, E. 2003. Amphiphilic biopolymers (amphibiopols) as new surfactants for membrane protein solubilization. *Protein Sci.* **12:** 681–689.

Ladavière, C., Tribet, C., and Cribier, S. 2002. Lateral organization of lipid membranes induced by amphiphilic polymer inclusions. *Langmuir* **18:** 7320–7327.

Nagy, J.K., Kuhn Hoffmann, A., Keyes, M.H., Gray, D.N., Oxenoid, K., and Sanders, C.R. 2001. Use of amphipathic polymers to deliver a membrane protein to lipid bilayers. *FEBS Lett.* **501:** 115–120.

Popot, J.-L., Berry, E.A., Charvolin, D., Creuzenet, C., Ebel, C., Engelman, D.M., Flotenmeyer, M., Giusti, F., Gohon, Y., Hong, Q., et al. 2003. Amphipols: Polymeric surfactants for membrane biology research. *Cell Mol. Life Sci.* **60:** 1559–1574.

Schuck, S., Honsho, M., Ekroos, K., Shevchenko, A., and Simons, K. 2003. Resistance of cell membranes to different detergents. *Proc. Natl. Acad. Sci.* **100:** 5795–5800.

Sihorkar, V. and Vyas, S.P. 2001. Potential of polysaccharide anchored liposomes in drug delivery, targeting and immunization. *J. Pharm. Pharmaceut. Sci.* **4:** 138–158.

Sunamoto, J., Sato, T., Taguchi, T., and Hamazaki, H. 1992. Naturally occurring polysaccharide derivatives which behave as an artificial cell wall on an artificial cell liposome. *Macromolecules* **25:** 5665–5670.

Thomas, J.L. and Tirrell, D.A. 1992. Polyelectrolyte-sensitized phospholipid vesicles. *Acc. Chem. Res.* **25:** 336–342.

Tribet, C. 1998. Hydrophobically driven attachments of synthetic polymers onto surfaces of biological interest: Lipid bilayers and globular proteins. *Biochimie* **80:** 461–473.

Tribet, C., Audebert, R., and Popot, J.-L. 1996. Amphipols: Polymers that keep membrane proteins soluble in aqueous solutions. *Proc. Natl. Acad. Sci.* **93:** 15047–15050.

Ueda, T., Lee, S.J., Nakatani, Y., Ourisson, G., and Sunamoto, J. 1998. Coating of POPC giant liposomes with hydrophobized polysaccharide. *Chem. Lett.* **5:** 417–418.